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GBV-C/HGV Infection in Chronic Hepatitis C Patients: Its Effect on Clinical Features and Interferon Therapy

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A novel virus (GBV-C/HGV) may be associated with some liver diseases including fulminant hepatitis and acute and chronic hepatitis. On the other hand, many investigations showed that this infection does not contribute to liver disease. GBV-C/HGV has been found to occur in association with infection with other hepatitis viruses. We investigated the effect of GBV-C/HGV infection on the clinical features and interferon treatment in patients with chronic hepatitis C. A total of 262 hepatitis C virus (HCV) RNA positive patients with chronic hepatitis were examined in this study. The detection of serum GBV-C/HGV RNA was done by RT-PCR using specific primers from the NS5 regions. Interferon-alpha was given at a dose of 6 MU/day for 16 or 24 weeks. A responder was defined as a patient with ALT normalization and HCV RNA disappearance after treatment.

GBV-C/HGV RNA was detected in 28 (11%) patients. No significant difference was detected in clinical features (age, sex, liver-related biochemical tests, and histological examination) between the 28 GBV-C/HGV-positive patients and the GBV-C/HGV-negative patients. Using interferon therapy for hepatitis C, the responder rates of GBV-C/HGV-positive and -negative patients were 14% and 20%, respectively. Of the 28 patients with GBV-C/HGV RNA, GBV-C/HGV RNA was tested after interferon therapy in 16 and of these GBV-C/HGV RNA was not detected in nine patients after therapy. These findings suggest that GBV-C/HGV infection dose not affect the clinical features in patients with HCV and the efficacy of interferon therapy for chronic hepatitis C. ***J. Med. Virol. 55:98–102, 1998.***

KEY WORDS: hepatitis C virus; GBV-C/HGV; coinfection; interferon

INTRODUCTION

Recently, a non A-E hepatitis agent, hepatitis GB virus C (GBV-C) was identified and shown to be a single-strand RNA virus of approximately 9.5 kilobases [Simons et al., 1995; Leary et al., 1996]. The hepatitis G virus (HGV) was cloned from the plasma of a patient with chronic hepatitis [Linnen et al., 1996]. HGV was shown to be closely related to GBV-C and associated with acute and chronic hepatitis [Zuckerman, 1996]. GBV-C/HGV has been detected in some patients with acute or chronic hepatitis, fulminant hepatitis, intravenous drug users, hemophiliacs, patients treated with hemodialysis, multiply transfused patients, and blood donors [Yoshiba et al., 1995; Zuckerman, 1996; Heringlake et al., 1996; Aikawa et al., 1996; Masuko et al., 1996]. Dual infection of GBV-C/HGV and other hepatitis viruses is not uncommon. Since GBV-C/HGV is transmitted by blood and blood products, patients with hepatitis C virus (HCV) seem to be at increased risk of contracting GBV-C/HGV [Zuckerman, 1996].

Coinfection of some hepatitis viruses modifies the clinical features of some viral liver diseases. For examples, simultaneous infection with hepatitis B virus (HBV) and hepatitis D virus (HDV) is often associated with severe viral liver disease such as fulminant hepatitis [Sherlock and Dooley, 1993]. Dual infection with

Abbreviations used: ALT, alanine aminotransferase; GBV-C, hepatitis GB virus C; HAI, histology activity index; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HGV, hepatitis G virus; RT-PCR, reverse transcription and polymerase chain reaction.

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HBV and HCV can result in inhibitory action between replicative levels of these viruses [Fong et al., 1991; Sheen et al., 1992; Pontisso et al., 1993; Ohkawa et al., 1994, 1995] and more frequent coinfection of HBV and HCV has been observed in advanced liver disease [Yuki et al., 1992].

We studied the prevalence of GBV-C/HGV and its effect on the clinical manifestations and interferon treatment in patients with chronic hepatitis C.

PATIENTS AND METHODS

Patients

Sera from 262 consecutive Japanese patients with chronic hepatitis C were used in this study. All patients had been positive for serum HCV RNA. The 178 men and 84 women ranged in age from 18 to 73 (mean ± SD = 52 ± 10) years. The HCV genome subtypes 1a, 1b, 2a, and 2b were found in 1, 194, 48, and 12 patients, respectively. In the remaining seven patients, the HCV genome subtype could not be classified. All serum samples were stored at –80°C for this study.

HCV RNA was extracted from the serum samples, copied into complementary DNA (cDNA) by reverse transcription, and amplified by polymerase chain reaction, as described previously [Hagiwara et al., 1992]. Serum HCV RNA levels were also evaluated with an amplicor HCV monitor which is a single-step, quantitative assay combining reverse transcription and polymerase chain reaction (RT-PCR) [Roth et al., 1996]. This assay was carried out according to the manufacturer's instructions. The limit of detection by the amplicor HCV monitor was 1.0×10^2 copies/ml. Typing of the HCV genome subtype was done by a method described previously [Mita et al., 1994] and named by Simmond's classification [Simmonds et al., 1994].

All patients underwent liver biopsy. The specimens were evaluated according to the histology activity index (HAI) scoring system [Knodell et al., 1981].

RT-PCR for Detection of HGV RNA

GBV-C/HGV RNA was extracted from serum samples, converted to cDNA, and subjected to the PCR assay. Briefly, RNA was extracted from 100 μl of serum sample with 300 μl of Trizol-LS reagent (Gibco-BRL, Gaithersburg, MD) and 80 μl of chloroform. The nucleic acids were coprecipitated with 10 μg of tRNA (Sigma, St. Louis, MO). The pellet was dissolved in TE buffer (10 mM Tris-Cl, pH 8.0; 1 mM ethylenediaminetetraacetate, pH 8.0), primed with 20 pmol of antisense primer, and then converted to cDNA using Superscript II (Gibco-BRL) according to the manufacturer's instructions. The products were subjected to the first amplification (45 cycles: 94°C for 1 min, 55°C for 1 min 15 sec, 72°C for 1 min; final extension at 72°C for 10 min) with five units of Taq polymerase (Takara Biochemicals, Kyoto, Japan) and 20 pmol of sense primer. The pair of primers used was derived from the putative NS5 region (77F, sense primer, 5′-CTCTTTGTGGTAGTAGCCGAGAGAT-3′; 211R, antisense primer, 5′-CGAATGAGTCAGAGGACGGGGTAT-3′; a kind gift from Dr. Jungsuh P. Kim, Genelabs Technologies, Inc.) [Linnen et al., 1996]. A 20-μl aliquot of the PCR products was separated on 1.5% agarose gel. Visualization was done with ethidium bromide under ultraviolet illumination, and the sample was transferred to a nylon membrane (Hybond-N$^+$; Amersham, Buckinghamshire, England). The membrane was hybridized at 55°C overnight with the ^{32}P-labeled oligonucleotide (152F, sense, 5′-TCGGTTACTGAGAGCAGCTCAGATGAG-3′) which stands between the primers used for RT-PCR. The membrane was then washed in 0.1 × standard saline citrate/0.1% sodium dodecyl sulfate and exposed overnight at –80°C to Kodak XAR-2 X-ray film (Kodak, Rochester, NY) with intensifying screens. For further confirmation of PCR products, we also undertook liquid hybridization. PCR products, 10 μl each, were incubated with the ^{32}P-labeled oligonucleotide (152F) at 95°C for 5 min and 55°C for 15 min. After electrophoretic separation on 12% polyacrylamide gel, the gel was exposed to X-ray film with intensifying screens.

Interferon Therapy

Patients received natural interferon-alpha (human lymphoblastoid interferon; Sumitomo Pharmaceuticals Co., Osaka, Japan) at a dose of 6 million units a day for 2 weeks and then three times per week for 14 or 22 weeks. The efficacy of the interferon treatment was evaluated according to the change in ALT levels and virological response. Responders were defined as patients who were without HCV RNA and with normal ALT levels, within 6 months after the end of interferon treatment, and remained so for a further 6 months.

Statistical Analysis

Statistical analysis was carried out using the Chi-square test and Student's *t*-test. Wilcoxon's rank-sum test was also used for the group comparison of HCV levels. Any *P* value less than 0.05 was considered statistically significant.

RESULTS

Clinical Features of Chronic Hepatitis C With and Without GBV-C/HGV RNA

GBV-C/HGV RNA was found in 28 (11%) of the 262 patients with chronic hepatitis C; 23 men and 5 women ranging in age from 28 to 63 (48 ± 10) years. With respect to the HCV genotype, GBV-C/HGV RNA was detected in 25 (13%) of the 194 HCV genotype 1b patients, two (4%) of the 48 HCV genotype 2a patients, and one (8%) of the 12 HCV genotype 2b patients. Table I shows the clinical characteristics (age, sex, previous blood transfusions, liver-related biochemical tests, and histological examination) of the chronic hepatitis C patients with and without GBV-C/HGV RNA. No significant difference was observed between HGV-positive and -negative patients with chronic hepatitis C.

To investigate the effect of GBV-C/HGV infection on

TABLE I. Clinical and Biochemical Characteristics of Patients With Hepatitis C*

Clinical features	GBV-C/HGV-positive	GBV-C/HGV-negative	Differences
Number of patients	28	234	
Age (yr)	48 ± 10 (28–63)	52 ± 11 (18–73)	n.s.
Female/Male	5/23	79/155	n.s.
Blood transfusion	26%	34%	n.s.
HCV genome subtype (1a:1b:2a:2b: unclassified)	0:25:2:1:0	1:169:46:11:7	n.s.
Serum levels			
ALT (IU//L)	122 ± 145	116 ± 91	n.s.
AST (IU/L)	75 ± 78	81 ± 62	n.s.
γGTP (IU/L)	60 ± 41	74 ± 65	n.s.
Bilirubin (mg/dl)	0.8 ± 0.3	0.8 ± 0.3	n.s.
Albumin (mg/dl)	4.1 ± 0.3	4.0 ± 0.5	n.s.
Histological examination			
HAI score	9.1 ± 3.4	9.3 ± 4.1	n.s.
I	2.4 ± 1.9	2.6 ± 1.8	n.s.
II	2.0 ± 1.2	2.1 ± 1.2	n.s.
III	2.5 ± 1.0	2.5 ± 1.0	n.s.
IV	2.3 ± 1.2	2.1 ± 1.1	n.s.

*Values are expressed as mean ± SD. n.s., Not significant; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGTP, γ glutamyltranspeptidase; HAI, histology activity index.

HCV replication, serum HCV RNA levels were measured in 27 of the 28 HGV-positive patients and in 218 of the 234 GBV-C/HGV-negative patients using the amplicor HCV monitor are shown in Figure 1. The HCV RNA levels of the GBV-C/HGV-positive and -negative patients did not differ significantly (median HCV RNA level 2.5×10^5 copies/ml vs. 1.0×10^5 copies/ml). In HCV genotype 1b patients, the HCV RNA levels were not different between the two groups (median HCV level 3.0×10^5 copies/ml vs. 1.0×10^5 copies/ml).

Influence of GBV-C/HGV Infection on Interferon Therapy for Chronic Hepatitis C

Four (14%) of the GBV-C/HGV-positive patients and 47 (20%) of the GBV-C/HGV-negative patients were responders. In HCV genotype 1b patients, four (16%) of the 25 GBV-C/HGV-positive patients and 18 (11%) of the 169 GBV-C/HGV-negative patients were responders. The rates of the responders did not differ between the two groups.

Of the 28 GBV-C/HGV-positive patients, GBV-C/HGV RNA at 3 or 6 months after the end of interferon therapy was examined for 16 cases. In nine (56%) of them, GBV-C/HGV RNA was not detected. The ALT response during interferon therapy in these patients is shown in Figure 2. Normalization of ALT levels after interferon therapy was observed in patients with clearance of HCV.

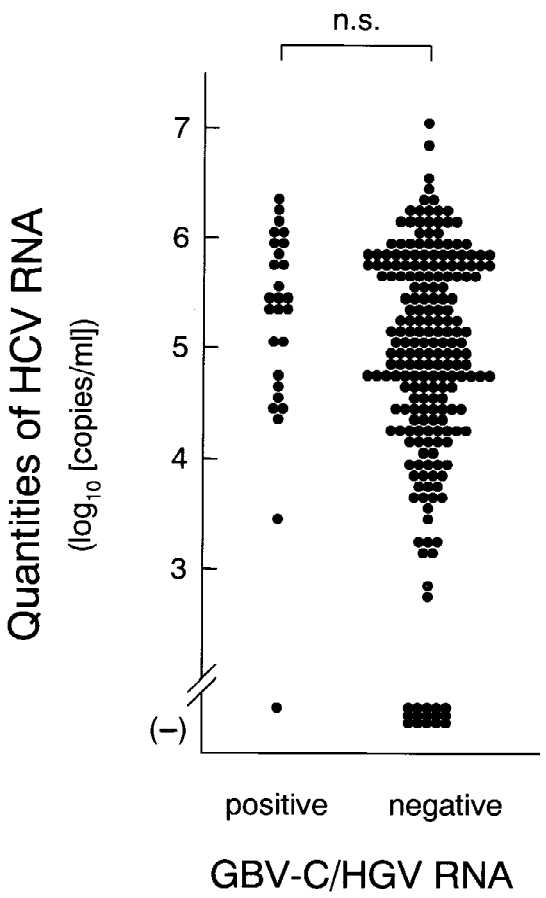

Fig. 1. HCV RNA levels in patients of chronic hepatitis C with or without GBV-C/HGV RNA. HCV RNA was measured with an amplicor HCV monitor. n.s., not significant.

DISCUSSION

Coinfection with multiple hepatitis viruses is reported to change the characteristics of liver disease caused by a single hepatitis virus. In advanced liver disease, frequent coinfection with HBV and HCV has been observed [Yuki et al., 1992]. Coinfection with HBV, HCV, and HDV and of HBV and HCV is associated with histologically more severe liver disease than HCV alone [Weltman et al., 1995]. Thus, in multiple hepatitis virus infection, a possible conjunctive role of the viruses has been suggested in the progression of liver disease. However, in the present study, the liver-related function test and histological examination results evaluated from the histological activity index, were not different between patients coinfected with HGV and HCV and with HCV alone (Table I). In liver cirrhosis, more frequent coinfection with HCV and HGV was not observed (data not shown).

In relation to viral replication, a suppressive action between hepatitis viruses has been reported in the infection with these viruses. Chronic hepatitis B patients with the antibody to HCV have lower serum HBV-associated DNA polymerase activity and higher incidence of hepatitis B surface antigen clearance than

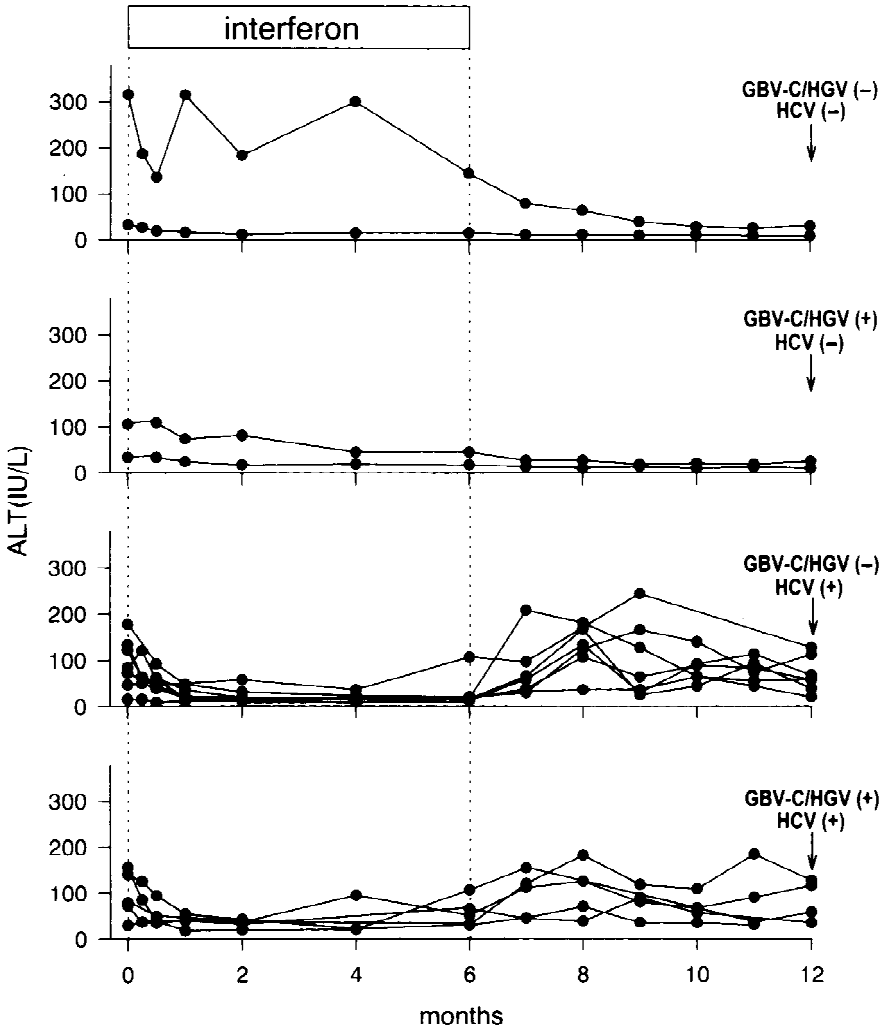

Fig. 2. ALT response to interferon therapy in patients of chronic hepatitis C with GBV-C/HGV. Of the GBV-C/HGV-positive patients, GBV-C/HGV RNA after the end of interferon therapy was examined for 16 cases. The individual lines represent the ALT pattern in individual patients when receiving interferon treatment. The ALT pattern was shown according to the disappearance of HCV and GBV-C/HGV at 6 months after the end of interferon therapy or not.

chronic hepatitis B patients without antibody to HCV [Fong et al., 1991; Sheen et al., 1992; Pontisso et al., 1993; Ohkawa et al., 1994, 1995]. However, no interference was found between HCV and GBV-C/HGV. GBV-C/HGV coinfection had no clear effect on the intensity of HCV replication as the serum HCV levels in GBV-C/HGV-positive subjects were equal to those in GBV-C/HGV-negative subjects (Fig. 1). As for HCV genotype, genotype 1b was shown to be more frequent in patients with HCV coinfection [Berenguer et al., 1996]. This tendency was recognized in our study, although not with statistical significance (the rates of HGV coinfection in 1b, 2a, and 2b patients were 13%, 4%, and 8%, respectively).

The usefulness of interferon treatment for chronic hepatitis C has been established [Davis et al., 1989; Di Bisceglie et al., 1989] and it is used widely. Several features including the HCV genome subtype and pretreatment HCV RNA levels, are reported to be predictive factors of the response to interferon treatment. Its efficacy has been reported to be lower in patients infected with HCV and other viruses such as HBV, HDV, and human immunodeficiency virus than in those with HCV alone [Weltman et al., 1995]. However, this study showed that GBV-C/HGV infection did not affect the efficacy of interferon treatment. With interferon treatment of patients with HCV and GBV-C/HGV, HCV was eradicated in all patients with ALT normalization. Of the nine patients who were GBV-C/HGV RNA-negative after interferon therapy, only two who were without HCV RNA had ALT normalization (Fig. 2). Thus, eradication of GBV-C/HGV was not involved in ALT normalization after interferon treatment in patients with HCV and GBV-C/HGV.

In conclusion, it was demonstrated that clinical manifestation, HCV replication and the efficacy of interferon therapy in patients with chronic hepatitis C are not affected by GBV-C/HGV infection. A role for

GBV-C/HGV in liver damage was not found in patients with chronic hepatitis C. Bralet et al. [1997] suggested that GBV-C infection does not affect histologic severity in chronic hepatitis C. Berenguer et al. [1997] showed that there is no association between the presence of HGV coinfection and the severity of liver disease in patients with end-stage HCV disease. However, recently, a specific strain of GBV-C/HGV has been shown to be involved in fulminant hepatic failure [Heringlake et al., 1996]. Further studies of the GBV-C/HGV genotype are needed to elucidate the role of GBV-C/HGV in liver damage.

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